Transport according to GARP: receiving retrograde cargo at the *trans*-Golgi network

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Tethering factors are large protein complexes that capture transport vesicles and enable their fusion with acceptor organelles at different stages of the endomembrane system. Recent studies have shed new light on the structure and function of a heterotetrameric tethering factor named Golgi-associated retrograde protein (GARP), which promotes fusion of endosome-derived, retrograde transport carriers to the *trans*-Golgi network (TGN). X-ray crystallography of the Vps53 and Vps54 subunits of GARP has revealed that this complex is structurally related to other tethering factors such as the exocyst, the conserved oligomeric Golgi (COG) and Dsl1 (dependence on SLY1–20) complexes, indicating that they all might work by a similar mechanism. Loss of GARP function compromises the growth, fertility and/or viability of the defective organisms, emphasizing the essential nature of GARP-mediated retrograde transport.

Introduction

Transfer of biomolecular cargo (e.g. proteins, lipids and other macromolecules) between organelles of the endomembrane system (e.g. the endoplasmic reticulum, Golgi apparatus, plasma membrane, endosomes and lysosomes) occurs by budding of vesicular or tubular transport carriers (TCs) from a donor compartment, followed by fusion of the TCs to an acceptor compartment [1]. Budding is carried out by a complex molecular machinery that is recruited from the cytosol to the acceptor membrane in the form of a protein 'coat' [2], whereas fusion is effected by a set of small, membrane-bound proteins named soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptors (SNAREs) [3]. Cognate SNAREs from both TCs (i.e. vesicle SNAREs; v-SNAREs) and the acceptor compartment (i.e. target SNAREs; t-SNAREs) assemble into a tight bundle of four α-helices, which brings the two membranes into close apposition, eventually leading to the merger of the lipid bilayers [3,4]. Additional regulators, including small GTPases [5] and their effectors, tethering factors [6,7] cooperate with the SNAREs in this process. Tethering factors are large proteins or protein complexes that establish long-range interactions between the TCs and the acceptor compartment before contacts between v-SNAREs and t-SNAREs occur, and that subsequently function to promote SNARE complex assembly [6,7]. The specificity and efficiency of each transport step in the endomembrane system are determined by the use of a particular combination of SNAREs, small GTPases, and tethering factors.

Tethering factors are classified into two types: homodimeric long coiled-coil proteins, and heteromeric multisubunit tethering complexes (MTCs) [6–8] (Box 1). Among the MTCs, there is a group of structurally related complexes named CATCHR (complex associated with tethering containing helical rods), which includes Dsl1 (dependence on SLY1–20), the conserved oligomeric Golgi (COG), the exocyst, and the Golgi-associated retrograde protein/Vps fifty-three (GARP/VFT) complexes [7] (Box 1). The study of tethering factors is a very active area of research in cell biology, and has been the subject of several reviews [6–8], but the understanding of the structure and function of GARP/VFT (hereafter referred to as GARP) has lagged behind that of other tethering factors. After the discovery of this complex in the yeast *Saccharomyces cerevisiae* a decade ago [9,10], its study went into a lull, and only recently has interest resurged with the description and characterization of the orthologous complex in higher eukaryotes [11,12]. Many new studies offer the first glimpses into the three-dimensional structure of GARP and highlight the crucial importance of this complex for a broad range of cellular functions. In this review, we focus on these recent developments in the understanding of GARP structure and function.

GARP subunit composition and function in retrograde transport

The discovery of GARP stemmed from genetic and biochemical studies of protein traffic in *S. cerevisiae* [9,10,13,14]. These studies established that GARP is a complex of four distinct proteins, termed vacuolar protein sorting 51 (Vps51), Vps52, Vps53 and Vps54 (Table 1). GARP is peripherally associated with the cytosolic face of the late Golgi apparatus (hereafter referred to as the *trans*-Golgi network; TGN), where it functions to tether 'retrograde' TCs derived from endosomes (Figure 1a) [9,10,13,14]. The ensuing TC–TGN fusion allows retrieval of recycling transmembrane

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Box 1. Classification of tethering factors (TFs) according to their structure.

This classification is based on [6–8] (Figure I). Only four of ~20 known golgins [8] are listed. TRAPP occurs as three related complexes (I, II and III) that differ in the identity of some of its subunits [6,7]. HOPS and CORVET also have common and distinct subunits [7]. The subunits of the four CATCHR complexes are structurally related but distinct [7]. CATCHR complexes were previously referred to as 'quatrefoil' because of the four-fold nature of some of them [18]; this latter term applies more to GARP than to other MTCs (see Figure 1). Most of these tethering factors are known to function as effectors of small GTPases of the Rab, Arl and/or Rho/Cdc42 families [6–8]. Thus, they preferentially bind to the GTP-bound (active) forms of the GTPases. By contrast, TRAPP complexes function as guanine nucleotide exchange factors (GEFs) for the Rab family GTPases, Ypt1 and Ypt31/32 [85].

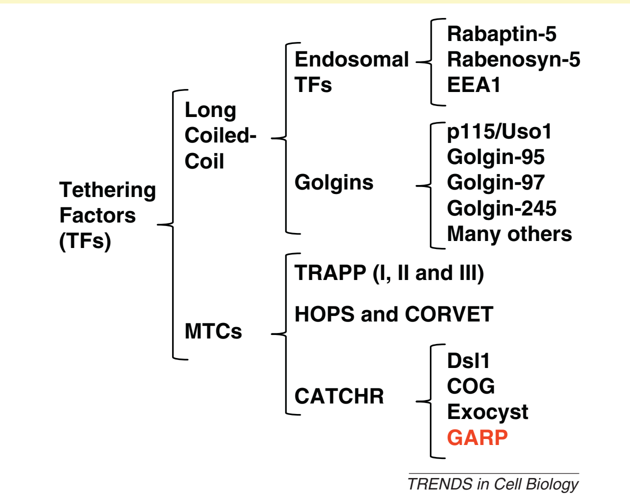

Figure I. Classification of TFs according to structure.

proteins such as the vacuolar protein sorting receptor Vps10 to the TGN. Vps10 binds newly synthesized vacuolar hydrolase precursors such as pro-carboxypeptidase Y (pro-CPY) at the TGN and takes them to endosomes, from where the hydrolase precursors go to the vacuole (the equivalent of the mammalian lysosome), while the receptors return to the TGN for further rounds of sorting [15]. Mutations in the genes encoding any of the four GARP subunits prevent recycling of Vps10 to the TGN, and consequently cause mis-sorting of the vacuolar hydrolase precursors to the exterior of the cell [9,10,13,14,16]. This phenotype is the basis for the designation of the four GARP subunits as 'Vps' proteins. Other recycling transmembrane proteins such as the dibasic endoprotease Kex2 [9,13] and the v-SNARE Snc1 [13,14,16] also require GARP for retrieval to the TGN. Their mis-sorting in GARP-deficient yeast strains results in additional phenotypic defects.

Recent studies have shown that the structure and function of GARP are conserved in higher eukaryotes. Orthologs of Vps52, Vps53 and Vps54 were readily identified by sequence homology to the yeast proteins and were also shown to assemble into a complex [11,12] (Table 1). Vps51 orthologs in higher eukaryotes were more difficult to identify because they exhibit low sequence identity to the yeast proteins. It was only during this past year (2010) that a human Vps51 ortholog was identified in a two-hybrid screen using human Vps53 as bait [17]. Remarkably,

Table 1. GARP subunit names and aliases

Organisms	GARP subunit names and aliases[1]			
Canonical	Vps51[2]	Vps52	Vps53	Vps54[3]
S. cerevisiae	Vps67, Whi6[4], Api3	Sac2		Luv1, Cgp1, Tcs3
A. thaliana		Pok	Hit1	
Drosophila				Scat
Zebrafish	Ffr			
Mammals	Ang2[5]	Are1[5], Sacm2l		Hcc8

[1]Most of the names and aliases of the GARP subunits are indicative of the broad range of defects that result from mutations in different model organisms, as follows: Api3, apical growth defects 3 [72]; Cgp1, centromere and promoter factor 1 (Cpf1) genetically interacting protein 1 [43]; Ffr, fat-free [73]; Hcc8, hepatocellular carcinoma 8 [11]; Hit1, heat intolerant 1 [74]; Luv1, loss upsets vacuole 1 [20]; Pok, poky pollen tube [55]; Sac2, suppressor of actin 2 [50]; Sacm2l, suppressor of actin mutation 2-like [75]; Scat, scattered spermatid nuclei [57]; Tcs3, temperature-sensitive clathrin synthetic 3 [76]; Vps, vacuolar protein sorting [77].
[2]Cloning and sequencing of *S. cerevisiae* Vps51 [14,13,79] revealed that it was allelic to the previously reported Vps67 [51] (systematic name: Ykr020w).
[3]*S. cerevisiae* Vps54 was also dubbed Rki1 [52], but in the meanwhile this name was assigned to a different protein.
[4]The term Whi (Whi6) denotes small cell size; it was coined to sound like Wee ('small') and to evoke the bottle of whisky that was won in a bet over the isolation of the corresponding *S. cerevisiae* mutants [59].
[5]Ang2 and Are1 are acronyms of 'another new gene 2' and 'a region expressed 1', respectively, reflecting the lack of knowledge of the functions of the corresponding proteins at the time of annotation [78,75]; Ang should not be confused with genes encoding angiogenin or angiopoietin, which have been given the same name.

human Vps51 turned out to be Ang2 [17], an uncharacterized open reading frame that had been previously recognized as being remotely homologous to subunits of other CATCHR complexes [18] and is orthologous to the zebrafish Fat-free (Ffr) protein [19] (Table 1). Biochemical analyses demonstrated that this protein is indeed part of an obligatory 1:1:1:1 complex of ~360 kDa with human Vps52, Vps53 and Vps54 (i.e. human GARP) [17].

Like its yeast counterpart, human GARP is mainly associated with the TGN (Figure 2a–c), although the existence of an endosomal pool has also been reported [11,12,17,19]. Human GARP participates in the retrieval of receptors for lysosomal hydrolase precursors, such as the mannose 6-phosphate receptors (MPRs), from endosomes to the TGN [12,17]. Accordingly, depletion of GARP subunits by RNA interference (RNAi) results in secretion of hydrolase precursors such as pro-cathepsin D into the extracellular space, with consequent lysosomal dysfunction [12,17]. GARP depletion also prevents retrograde transport of the TGN-resident protein TGN46, some SNAREs and the internalized Shiga toxin B-subunit (Figure 2d,e) from endosomes to the TGN. Thus, GARP plays a general role in endosome to TGN transport in a wide range of eukaryotes.

Insights into GARP structure

The four subunits of GARP are large proteins composed of 700–1700 amino acid residues in most eukaryotes (Figure 3a). An exception is Vps51 from *S. cerevisiae* and other yeasts, which comprises 125–320 residues distantly related to the amino-terminal region of Ang2. A common feature of the amino-terminal regions of all GARP subunits is the presence of short sequence stretches that are predicted to form coiled coils or amphipathic α-helices [9,11,13,14,17,18,20] (Figure 3). These structural elements have been proposed to participate in assembly of the complex, which indeed depends on the amino-terminal but not the carboxy-terminal (CT) regions of the GARP subunits [12,14,16,17,21]. Based on these findings and other structural consid-

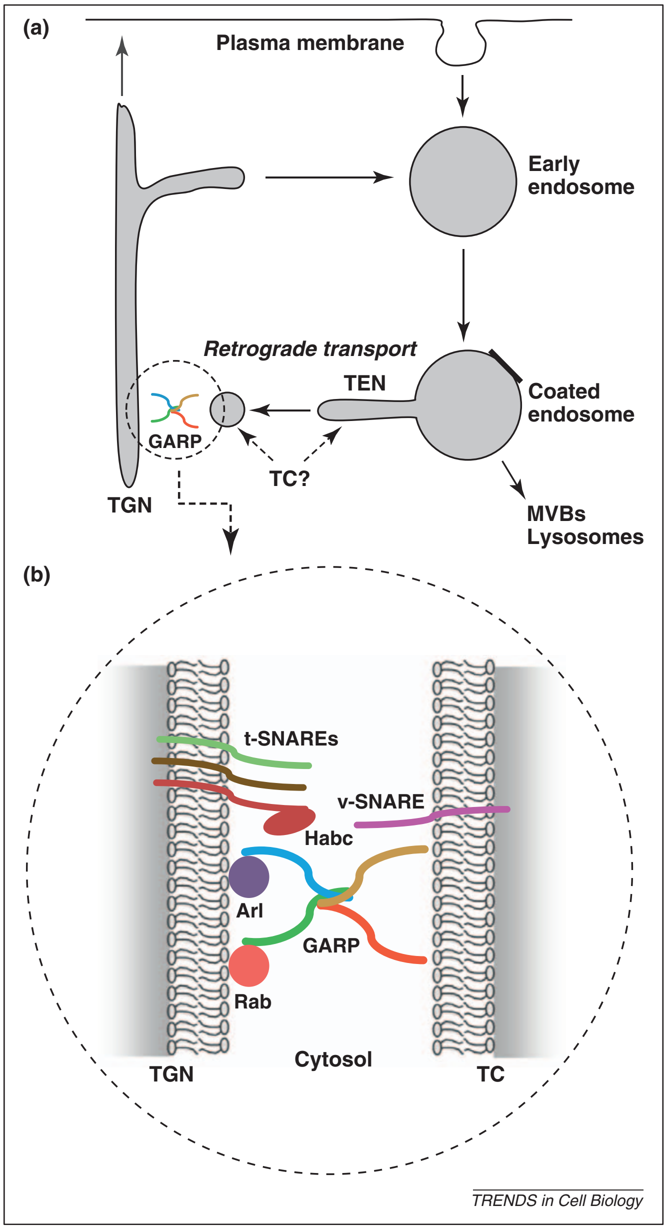

Figure 1. Proposed role of GARP in tethering retrograde transport carriers to the *trans*-Golgi network. **(a)** Protein cycling between the TGN and endosomes. Some transmembrane proteins, including acid hydrolase receptors, processing proteases and SNAREs, cycle between the TGN and endosomes [86,87]. Retrograde transport of these proteins from endosomes to the TGN occurs through a tubular compartment referred to as the tubular endosomal network (TEN). From this compartment, tubular or vesicular TCs deliver cargos to the TGN [86]. **(b)** Schematic representation of tethering mediated by GARP. GARP is shown as a heterotetramer assembled through the amino-terminal regions of its four subunits [12,14,16,17,21]. GARP has been shown to interact with small GTPases of the Rab and Arl families (Ypt6 and Arl1, respectively, in *S. cerevisiae*), which might contribute to GARP recruitment to the TGN [10,34]. GARP also interacts with the Habc domain of a t-SNARE (Tlg1 in *S. cerevisiae* and Syntaxin 6 in humans) [10,13,14,17,40], probably leading to displacement of this domain from the SNARE domain and thus enabling pairing with other SNAREs. Weaker interactions with other SNAREs [11,21] might further promote SNARE complex formation. The specifics of this graphic representation are highly speculative, because the molecular details of the interactions have not yet been elucidated.

erations, GARP is thought to consist of a core composed of the amino-terminal regions of the subunits, with four projecting arms (three in the case of yeast) corresponding to the CT regions of the subunits (Figure 1b).

Early sequence analyses revealed a low degree of homology of the subunits of GARP to subunits of the Dsl1, COG and exocyst complexes [18,22], which are now grouped under the CATCHR title [7]. The homology is most significant in the amino-terminal regions, but also extends to the CT regions of the proteins [18,22]. The notion that these complexes are related has been confirmed by the recent resolution of the crystal structures of CT fragments from human Vps54 [23] and *S. cerevisiae* Vps53 [24] (Figure 3b). These structures show that both proteins have an α-helical bundle organization similar to that of other CATCHR complex subunits [23,24], providing further evidence for their divergent evolution from a common ancestral molecule [7]. The entire length of CATCHR subunits is structured as an elongated array of α-helical bundles [7] (Figure 3b), which have been denoted A–E in the two largest CATCHR structures, Exo70 [25] and Dsl1 [26]. Based on structural comparisons, this domain nomenclature has been extended to other CATCHR structures [27,28,17]. The crystallized region of Vps54 folds as a compact bundle of five α-helices [23], which is most similar to the D-like domain of the exocyst Sec6 subunit [29]. Similarly, the crystallized region of Vps53 folds into two continuous α-helical bundles, similar to the D- and E-like domains of Sec6 [29]. The presence of a hydrophobic groove formed by the first two α-helices of the D-like domains in both crystal structures, in conjunction with sequence analyses, hints at the extension of the α-helical bundle organization towards the amino-termini of the proteins [23,24], as in Tip20 (Figure 3b). Sequence analyses predict that Vps51 and Vps52 are strongly α-helical, suggesting that they too might share a CATCHR fold.

The CATCHR fold is also found in the cargo-binding domain of the *S. cerevisiae* molecular motor Myo2, which tethers transport vesicles to actin filaments [30]. In addition, the MUN domain of MUNC13, which regulates SNARE complex assembly at the synapse [31], has been predicted to have a CATCHR fold based on remote sequence homology [32]. It is thus tempting to speculate that this fold might have evolved to enable vesicle tethering and SNARE complex assembly in a variety of contexts.

Despite these structural similarities, CATCHR complexes differ in the number of subunits and α-helical bundles, and in the curvature and flexibility of each subunit [7]. Furthermore, some subunits exhibit distinct structural elements such as hinges or disordered regions [28], which might endow the proteins with specific mechanistic properties. CATCHR complexes hence exemplify the adaptation of a common structural blueprint to the performance of distinct vesicle tethering events.

GARP interactions with membranes, small GTPases and SNAREs

GARP must exert its tethering function by simultaneously binding to both the TC and acceptor membranes. In this regard, the CT region of human Vps53 was shown to be required for binding of GARP to retrograde TCs and for retrieval of TGN46 to the TGN [21]. This is in line with the finding of a conserved patch of charged, polar and hydrophobic residues in the CT domain of *S. cerevisiae* Vps53 (Figure 4a, yellow), which is required for Vps10-mediated sorting of pro-CPY to the vacuole [24]. In addition, the CT region of *S. cerevisiae* Vps54 was found to bind to endosomes (the donor compartment for retrograde TCs) and to

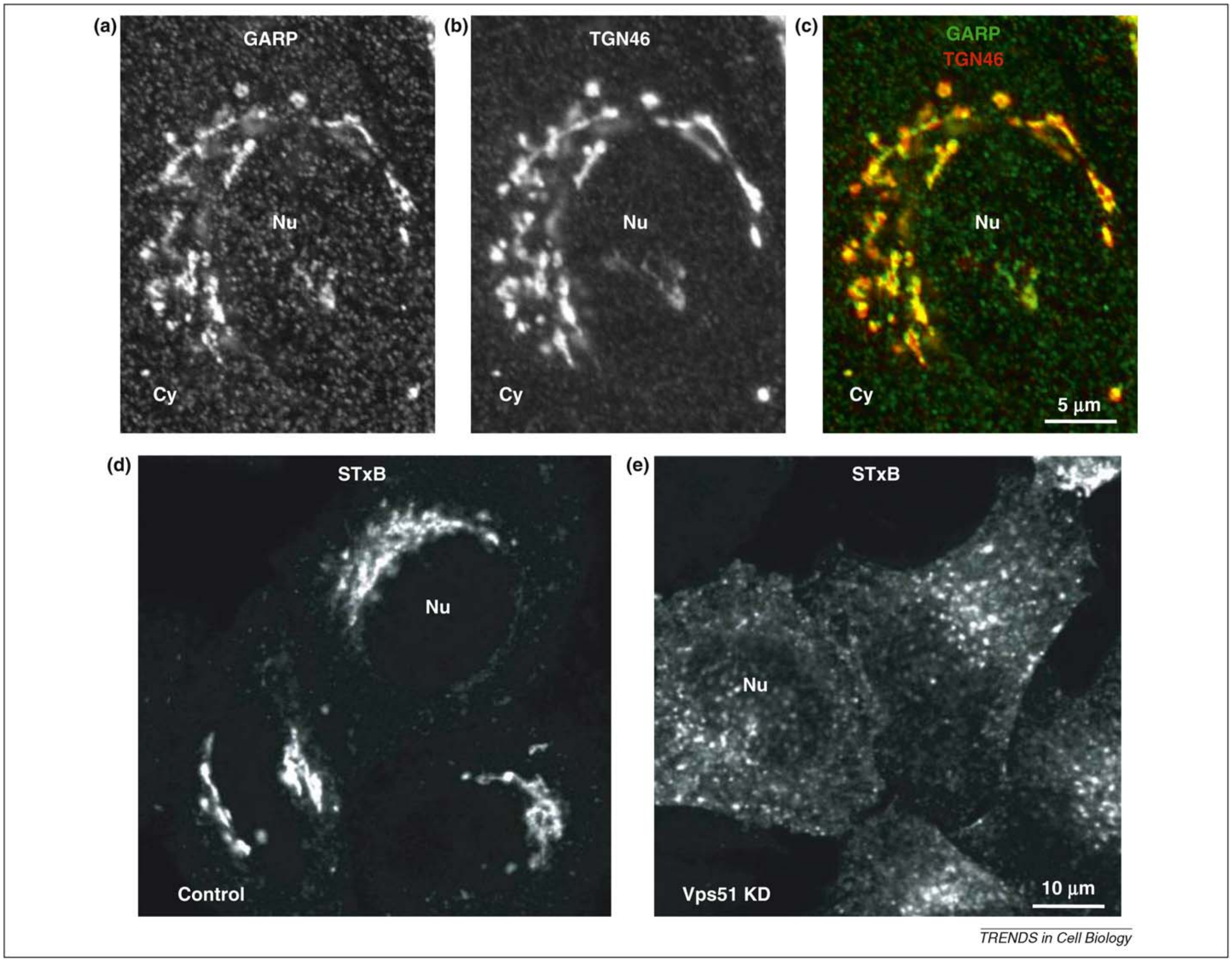

Figure 2. GARP localizes to the TGN, where it enables cargo transport from endosomes. **(a–c)** Co-localization of GARP (labeled by expression of a Vps54/green fluorescent protein chimera) (green channel) with the TGN marker TGN46 (labeled with a specific antibody to this protein followed by Alexa-594-conjugated secondary antibody) (red channel) in human HeLa cells under confocal fluorescence microscopy. **(c)** Merged image in which yellow indicates colocalization. The TGN appears as a cisternal/tubular network partially surrounding the nucleus (Nu). Cy, cytoplasm. **(d,e)** Evidence for the involvement of GARP in retrograde transport. Under confocal fluorescence microscopy, the internalized Cy3-conjugated B subunit of Shiga toxin (STxB) can be seen to reach the TGN in control HeLa cells, but accumulates in endosomes and retrograde transport carriers in HeLa cells depleted of Vps51 (i.e. Ang2) by RNAi knockdown (KD). Images are reproduced from **(a–c)** [12] and **(d,e)** [17].

participate in retrieval of Snc1 to the TGN [16]. The nature of the ‘receptors’ (e.g. proteins and/or lipids) for GARP on TCs and endosomes remains to be determined. The search for GARP-interacting proteins [33] might be an avenue to identify such receptors.

By contrast, binding of GARP to the acceptor membrane (i.e. the TGN) is probably mediated by interactions with GTPases of the Rab and Arl families (Figure 1b), as is the case for other tethering factors [6–8]. *S. cerevisiae* GARP interacts through its Vps52 subunit with the GTP-bound form of Ypt6, the ortholog of mammalian Rab6 [10,14], and through its Vps53 subunit with the GTP-bound form of Arl1 [34]. Depletion of Ypt6 causes dispersal of GARP from the TGN, as observed by immunofluorescence microscopy [10], but does not diminish its association with a membrane fraction in subcellular fractionation experiments [13]. Depletion of Arl1 does not alter GARP localization to the TGN [34]. It is therefore unclear whether Ypt6 and Arl1 regulate actual recruitment of GARP to the TGN or regulate some other function of the complex. Like GARP, the exocyst complex interacts with the GTP-bound form of two small GTPases, Rho3 and Cdc42 [35]. Loss-of-function mutations of these GTPases block exocytosis without altering exocyst localization [36–38]. It is thus apparent that these small GTPases regulate properties other than (or in addition to) the association of CATCHR complexes with their corresponding membranes. Membrane association might therefore depend on multiple interactions, only some of which are provided by small GTPases [39].

S. cerevisiae Vps51 specifically interacts with the amino-terminal regulatory Habc domain of the t-SNARE, Tlg1 [13,14]. In this interaction, the amino-terminal region of Vps51 (residues 9–30) forms a partial α-helix that binds to a hydrophobic groove on the three-α-helix bundle of the Habc domain [40]. This interaction appears to be phylogenetically conserved, because human Vps51 (i.e. Ang2) interacts with the Habc domain of Syntaxin 6 (i.e. the human ortholog of Tlg1) [17]. However, point mutations that disrupt the Tlg1–Vps51 interaction do not cause any trafficking defects in yeast [40], suggesting that additional

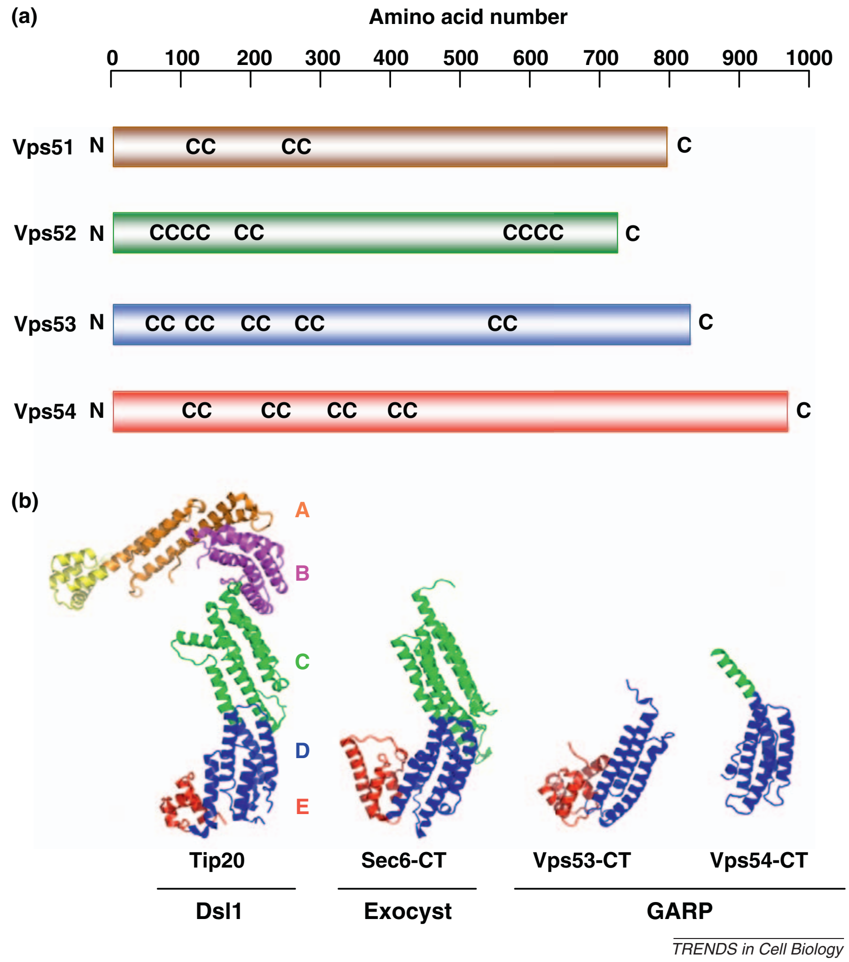

Figure 3. Characteristics of the GARP subunits. **(a)** Schematic representation of the human GARP subunits. N and C represent the amino- and carboxy-termini of the proteins. The scheme shows the approximate sizes of the four subunits and the location of predicted coiled-coil (CC) motifs. The presence of coiled coils, particularly in the amino-terminal regions, is a conserved feature of GARP subunits from all species analyzed. Analyses using the SMART server (http://smart.embl-heidelberg.de/) predict the presence of a C2H2-type zinc finger at the amino-terminus of Vps54 from *Drosophila* and *Caenorhabditis elegans* but not from other species. These domains generally function as binding sites for other macromolecules, but their exact role in these Vps54 orthologs is unknown. **(b)** Crystal structures of CT fragments from *S. cerevisiae* Vps53 [24] and human Vps54 [23] compared with those of a CT fragment from the *S. cerevisiae* Sec6 subunit of the exocyst complex [29] and the full-length *S. cerevisiae* Tip20 subunit of the Dsl1 complex [26]. Structures are represented as ribbon diagrams with the tandem α-helical-bundle domains (designated A–E) shown in different colors.

interactions might contribute to function. In this regard, the amino-terminal regions of human Vps53 and Vps54 bind to the SNARE motifs of other SNAREs involved in endosome to TGN transport, namely Syntaxin 6, Syntaxin 16 and Vamp4 [21]. Moreover, GARP binds not only single SNAREs but also SNARE complexes [21]. Finally, depletion of GARP reduces formation of TGN SNARE complexes [21]. Thus, GARP engages in various interactions with TGN SNAREs, contributing to their assembly and/or stabilization. The COG complex has also been shown to interact with the fusion machinery, including direct contact of Cog4 with the *cis*-Golgi t-SNARE Sed5p [41] and enhancement of the stability of intra-Golgi SNARE complexes [42]. Likewise, Dsl1 interacts with the N-terminal regulatory domains of ER SNAREs, although this interaction exerts only a modest effect on SNARE complex formation *in vitro* [28].

Requirement of GARP for a broad range of cellular processes

Although it is now well known that the primary role of GARP is in the reception of endosome-derived TCs at the TGN, the effect of GARP mutations on single cells and multicellular organisms extends far beyond this particular step (Table 2). Some phenotypic defects probably result from impaired TGN, endosomal or vacuolar/lysosomal functions that are secondary to altered retrograde transport. In this category might be included cell wall defects [43,44], hypersensitivity to metal ions, toxic drugs and pH extremes [20] in *S. cerevisiae* GARP mutants, and defective lipid absorption and transport in enterocytes of the *fat-free* zebrafish Vps51 mutant [19]. GARP mutations in *S. cerevisiae* [45] and RNAi in cultured human cells [17] also cause defects in autophagy, the process by which cells target cytoplasmic organelles and particles for lysosomal degradation [46]. Altered autophagy could result from lysosomal dysfunction [17], or from a requirement for some components of the autophagy machinery such as Atg9 to cycle between the TGN and endosomes [47] or between mitochondria and pre-autophagosomal structures [45]. Another possibility is that GARP plays a direct role in autophagy, as is the case for the COG [48] and TRAPP [49] tethering complexes.

GARP has also been shown to be required for maintenance of actin filaments [43,50,51] and microtubules [43,52]

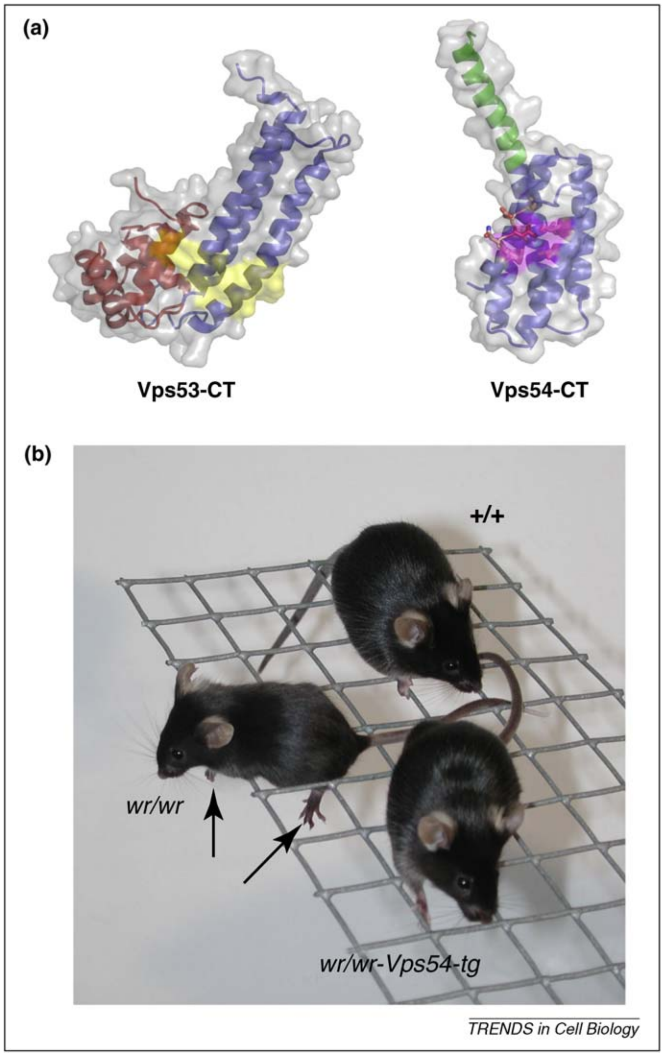

Figure 4. Functional regions of GARP subunits. **(a)** Ribbon diagrams of *S. cerevisiae* Vps53 and human Vps54 CT fragments shown under a translucent surface. Segments corresponding to the different α-helical-bundle domains are indicated in green (C domain), blue (D domain) and red (E domain). The yellow highlight on Vps53-CT indicates a cluster of highly conserved residues that are required for pro-CPY sorting to the vacuole [24]. The magenta highlight on Vps54-CT indicates a hydrophobic pocket containing the leucine-967 residue (red stick model) that is mutated in the wobbler mouse [23]. **(b)** Defective motor function of the Vps54-mutant wobbler (*wr*) mouse. Unlike a normal mouse (+/+), a homozygous wobbler mouse (*wr/wr*) cannot grab onto the grid with either the forelimbs or hindlimbs (arrows) as a result of motor neuron degeneration. Insertion of a normal Vps54 allele (*wr/wr-Vps54-tg*) into the genome rescues the phenotype of the mutant mouse. Photograph courtesy of Thomas Schmitt-John (Aarhus University, Denmark), reproduced with permission from [60].

in *S. cerevisiae*, although these requirements appear to be dependent on the genetic background of the strains [9,20,43]. Defects in the actin cytoskeleton or membrane traffic in GARP mutants might underlie the impaired polarity or formation of reproductive structures such as *S. cerevisiae* buds [53], pheromone-induced mating projections (shmoos) [43] and spores [54], *Arabidopsis thaliana* pollen tubes [55,56], and *Drosophila* sperm cells [57].

In light of the broad range of cellular processes that depend on GARP function, it is not surprising that mutations in GARP genes severely compromise the growth, fertility and/or viability of the mutant organisms. For example, *S. cerevisiae* GARP mutants are viable, but exhibit slow, temperature-sensitive growth [9,14,20] and small cell size [58,59]. Homozygous *Drosophila* GARP mutants have also been reported to be viable, although the mutants display reproductive and developmental defects [57]. GARP mutations are even more deleterious to plants and mice. Homozygous mutations in GARP genes are embryonically lethal in *A. thaliana* [55,56], and in the mouse, homozygous disruption of the gene encoding Vps54 results in abnormal development and lethality of embryos between days 11.5 and 12.5 post-gestation [60]. These findings emphasize the essential nature of GARP for many cellular functions.

Motor neuron degeneration caused by defective GARP

Of particular interest regarding the physiological requirement of GARP is the finding [60] that a single amino acid substitution (leucine-967 to glutamine) in the CT region of Vps54 is the cause of defective spermiogenesis and motor neuron disease in the 'wobbler' mouse (Figure 4b). This mouse strain arose as the result of a spontaneous, recessive mutation more than 50 years ago [61]. Characterization of the phenotype showed that homozygous mutant mice display unsteady ('wobbly') gait with progressive muscle weakness, atrophy and contractures, predominantly in the forelimbs, head and neck [62]. Severe muscle weakness is eventually fatal for some, although not all, of the affected mice [62]. This phenotype is caused by degeneration of motor neurons [62], and resembles the human disease, amyotrophic lateral sclerosis (ALS), also named Lou Gehrig's disease after the American baseball player who was one of its best-known victims [63]. A recent screening for mutations in the Vps54 gene in a cohort of patients with familial and sporadic forms of ALS identified a substitution of alanine for threonine-360 in only 1 out of 192 patients, ruling out mutations in this protein as a major cause of ALS [64].

The hydrophobic leucine-967 residue is completely buried against the CT α-helical bundle of Vps54 (Figure 4a) [23]. Its substitution by a hydrophilic glutamine residue in the wobbler mouse destabilizes the protein and causes it to be rapidly degraded [23]. As a consequence, levels of the mutant protein are greatly reduced in all tissues of the wobbler mouse, including the spinal cord, where many motor neurons are located [23]. Levels of Vps53 are similarly decreased, as a result of degradation of the excess unassembled protein, and reflect reduction of the whole GARP complex [23]. Evidently, the lower levels of GARP in the wobbler mouse are sufficient to support viability of the animal, in contrast to the embryonic lethality of the Vps54-null mouse [60], but not the long-term health of its motor neurons.

How does a GARP deficit specifically lead to motor neuron degeneration? Motor neurons have very long axons that innervate skeletal muscles far away from the cell body. Maintenance of this distinct architecture probably requires optimal function of the protein trafficking machinery. Partial defects that are tolerable to less sprawling cell types might have catastrophic consequences for far-flung neuronal processes. The broad influence of GARP deficiency on intracellular organelles and the cytoskeleton (Table 2) could affect the ability to transport cargos, in both retrograde and anterograde directions, along the axon. Indeed, abnormalities in axonal transport have been reported to be a major cause of motor neuron death [65], perhaps as a result of

Table 2. Requirement[1] of GARP for a broad range of cellular processes

Organisms	Processes	References
S. cerevisiae	Cell growth and viability	[9,20,43]
	Transport of Vps10 (vacuolar sorting receptor), Kex2 (dibasic endoprotease), Ste13 (dipeptidyl aminopeptidase A), Snc1 (exocytic v-SNARE) and Sso1 (plasma membrane t-SNARE) from endosomes to the late Golgi complex	[9,13,14,16,40]
	Sorting of hydrolase precursors to the vacuole	[9,13,20,51,76]
	Maintenance of vacuole integrity	[9,13,14,20,76]
	Pro-α-factor processing	[9,51,76]
	Positioning of the mitotic spindle at the neck between mother and daughter cell	[43]
	Maintenance of actin cytoskeleton integrity and polarization	[43,45,50,51]
	Polarized localization of the unconventional myosin motor protein Myo2	[43]
	Bipolar bud site selection	[53]
	Formation of pheromone-induced mating projections (shmoos)	[43]
	Cell wall composition and integrity	[43,44]
	Meiotic progression and spore formation	[54]
	Cytoplasm to vacuole targeting	[79]
	Autophagy	[45,79]
	Maintenance of the mitochondrial tubular network	[45]
	Resistance to high concentrations of Zn^{2+}, Mn^{2+} and Cd^{2+}, hygromycin B, caffeine, FK506, gentamicin, caspofungin, microtubule-destabilizing and actin-disrupting drugs, killer toxin 1. and extremes of pH	[20,43,52,80–82]
A. thaliana	Embryonic development and viability	[55,56]
	Gametophyte development	[55,56]
	Pollen tube apical growth	[55,56]
	Tolerance to heat and osmotic stress	[74]
Drosophila	Male fertility	[57]
	Sperm individualization from syncitial cysts	[57]
Zebrafish	Maintenance of lipid transport and Golgi morphology in the digestive tract	[19]
Mammals	Embryonic development and viability	[60]
	Retrograde transport of CI-MPR (acid hydrolase receptor), TGN46 (TGN protein), Vamp4 (v-SNARE), Syntaxin 6 (t-SNARE) and Shiga toxin B subunit from endosomes to the TGN	[12,17,21,23,33]
	Sorting of cathepsin D (acid hydrolase) to lysosomes	[12,17]
	Lysosome morphology and function	[12,17]
	Motor neuron survival	[60]
	Spermiogenesis	[60]
	Mitochondrial morphology and function	[83]
	Autophagy	[17]
	Endosomal cholesterol transport	[17]
	Infection of T cells by HIV-1	[84]

[1]Most of these requirements were determined in studies of the phenotypes of mutant organisms.

defective reception in the cell body of trophic signals coming from the nerve terminal through signaling endosomes [66]. Another clue to the pathogenesis of motor neuron disease in the wobbler mouse might lie in the autophagy defects caused by GARP deficiency. Some forms of hereditary ALS result from mutations in proteins such as copper/zinc superoxide dismutase 1 (SOD1) and TDP-43 (trans-activation response DNA binding protein 43), which cause these proteins to accumulate as ubiquitinated aggregates in the cytoplasm of motor neurons [63,67]. Forms of sporadic ALS also present with neuronal inclusions that correlate with the severity of the disease [68]. These inclusions could act as 'sinks', sequestering proteins that are essential for cell survival. Strikingly, wobbler motor neurons exhibit ubiquitinated TDP-43 inclusions similar to those in ALS [69]. It is thus tempting to speculate that the autophagy defect caused by reduced GARP levels in the wobbler mouse prevents effective clearance of cytoplasmic aggregates, eventually leading to motor neuron death.

Concluding remarks

Like the homonymous character in John Irving's novel [70], GARP is finally coming of age. Judging from the accelerating pace of discovery of its structure and function, we should soon have a more complete understanding of its mechanism of action. We still need to learn the details of the interactions of GARP with GTPases, SNAREs and other proteins, and to elucidate how exactly it participates in tethering and fusion at the TGN. Relevant to this mechanism is the question of whether GARP cooperates with other tethering factors in the performance of its function. It is intriguing that retrograde transport of several cargo molecules to the TGN is blocked by depletion of not only GARP but also several golgins [71]. The structures of GARP and the golgins are so different that it is difficult to imagine that they function in similar ways in the process of tethering. We also need to explain how GARP deficiency has such a broad influence on cell and organismal physiology, including the pathogenesis of neurodegenerative disorders. Does GARP perform other functions in addition to tethering TCs at the TGN? The elucidation of these and other outstanding issues will be enabled by further studies on GARP and other CATCHR complexes of related structure and function.

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